CHANGES IN BODY WEIGHT AND WATER CONSUMPTION IN BLACK-TAILED PRAIRIE DOGS AND ROCK DOVES FOLLOWING RED PHOSPHORUS SMOKE EXPOSURES

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*Effects of red phosphorus/butyl rubber (RP/BR) smoke on two wildlife species, black-tailed prairie dogs (*Cynomys ludovicianus*) and rock doves (*Columbia livia*), were evaluated in laboratory range-finding experiments. Prairie dog groups were exposed to 2.0, 4.0, or 6.0 mg/L target concentrations of smoke generated for 1 h over 1–4 daily exposure sessions. Rock dove groups were exposed to either 0.0, 3.0, or 6.0 mg/L target concentrations for comparable time periods. Animals were monitored for body weight and water consumption changes for 28 days after their last smoke-exposure day. Body weight losses were severe and protracted in male rock doves, and these were correlated with significant mortality rates. Male doves exposed to 6.0 mg/L smoke concentration level never recovered their lost body weights to preexposure levels during the 28-day observation period. Prairie dogs, in contrast, only showed a 1-day body weight loss postexposure and a rapid recovery to their preexposure weight levels. Both species showed depressed water intakes for 1–2 days, followed by significantly elevated, sustained water consumption levels on days 10–28 postexposure, with the higher consumption levels directly related to the total number of daily smoke exposure sessions in rock doves. These protracted periods of elevated water consumption late in postexposure could have been due to lung irritation, inflammation, and edema effects previously indicated in albino rat studies. In rock doves, physical obstruction of the respiratory passages by mucus and exudate associated with pulmonary irritation could have also led to increased exertion due to breathing difficulties, increased energy expenditure, and a subsequent need for high water intake levels during late postexposure.*

When burned, red phosphorus butyl rubber (RP/BR) generates a dense white smokescreen and is used to conceal military troop movements (Burton et al., 1982). The white smoke consists primarily of finely divided phosphoric and polyphosphoric acid particles, along with trace amounts of carbon monoxide gas. Human health effects and risks associated with the inhalation

Received 29 November 1993; accepted 18 February 1994.

This research was conducted as part of project order 85PP5847 between the Health Effects Research Division, U.S. Army Biomedical Research and Development Laboratory (USABRDL) and the Denver Wildlife Research Center (DWRC), Animal Damage Control (ADC), Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA). The Project Order was initiated with the support of the Fish and Wildlife Service, U.S. Department of Interior; the Center transferred to APHIS, USDA on March 3, 1986. Reference to trade names or commercial products does not constitute an endorsement by the Federal government.

We thank Ken Crane, Stanley Gaddis, and Brad Johns for technical assistance during conduct of the aerosol exposures; Paige Groninger for data transcriptions; Kathleen Fagerstone and Donald Elias for editorial comments; and Patricia Wittstruck for word-processing revisions of the manuscript.

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Inhalation Toxicology, 6:407–425, 1994

0895–8378/94 $10.00 + .00

of RP/BR smoke have been evaluated by the Health Effects Research Division, U.S. Army Medical Research and Development Command Laboratory at Fort Detrick, Frederick, Md.

Lethal effects of RP/BR combustion products have been evaluated in two previous investigations. Burton et al. (1982) exposed albino rats to a range of concentrations from 1.5 to 8.5 mg/L. Their data indicated a lethal concentration value (LC50) of 2.46 mg/L for 5 daily 1-h exposure sessions. Necropsy examinations indicated laryngeal and epiglottal injury at exposure levels above 5.0 mg/L. Signs observed in the rats included pulmonary congestion, edema, and hemorrhage. A second study, involving albino rats (Aranyi, 1983), indicated an LC50 value of 2.32 mg/L for 5 daily 1-h exposures.

Qualitative effects of RP/BR smoke exposure in prairie dogs and rock doves have been reported previously (Shumake et al., 1992). Prairie dogs showed no mortality postexposure to 2.0, 4.0, or 6.0 mg/L aerosol concentrations for 1–4 daily 1-h exposure sessions. Rock doves, however, showed 26% mortality within 8 days postexposure to 3.0 or 6.0 mg/L concentrations over 1–4 successive sessions, with male doves showing significantly higher mortality than females. Both doves and prairie dogs showed an increase in the number of affected or lost vocalizations postexposure. Body posture changes were observed in the doves, and an increase in respiratory congestion was noted in prairie dogs postexposure.

In terms of ecotoxicological effects, some research (Van Voris et al., 1986) has been conducted on the effects of RP/BR smoke exposures with various plant species. However, only a few published reports are available that are related to the inhalation effects of this material on wild animals (Johns et al., 1992; Shumake et al., 1992; Sterner, 1993). Concerns regarding the potential of repeated RP/BR smoke exposures on ecosystems established adjacent to or on military training installations led to the current study on black-tailed prairie dogs (*Cynomys ludovicianus*) and rock doves (*Columbia livia*). These species were selected based on their extensive overlapping ranges throughout the central United States, their availability, and their adaptability to the laboratory environment.

The purpose of this study was to determine some toxicological effects of RP/BR smoke inhalation exposures in concentration range-finding tests using body weight and water consumption changes as dependent measures for each species. The main independent variables were gender, concentration of smoke, and number of repeated exposures. Smoke exposure effects on body weight and water consumption were evaluated using repeated-measures analysis of variance (ANOVA) designs on data collected through day 28 postexposure. For both species, null hypotheses under test for each measure were essentially the same: (1) Smoke exposures would produce effects equal in strength and duration for both sexes; (2) different concentrations of smoke would produce effects equal in strength and duration; and (3) different numbers of smoke exposures would produce effects equal in strength and duration.

MATERIALS AND METHODS

Animals

Black-Tailed Prairie Dogs Prairie dogs were captured at Buckley Air National Guard Base, Aurora, Colo., by dispensing large volumes of soap and water into individual burrows (see Elias et al., 1974). After transport to the Research Center, the animals were dusted with pyrethrum-containing powder to control ectoparasites, weighed (nearest gram), and implanted with a subcutaneous transponder (Identification Devices, Inc., Boulder, Colo.) for individual identification (Fagerstone & Johns, 1987). Animals were held in individual cages and were fed Purina Rabbit Checkers ad libitum and fresh cabbage 3 times per week during both the 14-day quarantine period and a 14-day laboratory acclimation stabilization period. Animal rooms were maintained with temperature (23 ± 2°C) and light : dark (12 : 12 h forward) control throughout the experiment.

Rock Doves A shipment of 122 rock doves was purchased from a local supplier. Birds were captured in the north Denver area with cannon nets (Grubb, 1988). Birds were then placed in wire mesh outdoor aviary cages (3.0 × 1.5 × 1.8 m) and held for 13 wk. Up to 30 doves were held per cage, and the ad libitum maintenance diet consisted of Purina Pigeon Checkers, cracked corn, grit, and water. The doves were then moved to an indoor quarantine facility, an 11.5-m-diameter steel storage building, with heat and light provided. The birds were leg-banded with individual identification numbers, weighed, and dusted for ectoparasites. They were then checked for overt signs of poor health (Veterinary Services, APHIS, USDA) before being placed in three wire mesh aviaries. Animals were maintained on Purina Pigeon Checkers and water ad libitum in this facility on a 12 : 12 h forward light : dark cycle for 29 days.

In the main research building doves were held in individual cages in a separate temperature (23 ± 2°C) and light : dark (12 : 12 h) controlled room. The birds were fed the same diet as during quarantine throughout the experiment and were given 14 days of acclimation before tests were initiated.

Inhalation Exposure System

Two exposure chambers (Bertke and Young, Cincinnati, Ohio) constructed with stainless steel and of identical dimensions (91.5 × 91.5 × 132.0 cm) were used for simultaneously exposing 6 animals in each group to either filtered air or RP/BR smoke. Animals were held in individual stainless steel cages (30.5 cm^3) during each exposure session. Both exposure chambers were set up in separate adjacent rooms with separate air filtration and humidity control units. Chamber air flow rates were held constant at 250 L/min for all exposure sessions by separate industrial vacuum units (Dayton, model 3Z707A, Chicago) powered through variable 110-VAC rheostats (STACO Inc., Dayton, Ohio).

The RP/BR extruder and smoke aerosol generator equipment have been described previously in detail in other reports (Shumake et al., 1992; Sterner et al., 1991; Holmberg et al., 1985). Briefly, the RP/BR product, formulated at the Bio/Organic Analysis Section, Analytical Chemistry Division, Oak Ridge National Laboratory (ORNL), Oak Ridge, Tenn., was extruded slowly by an adjustable precision metering pump in order to generate specific concentrations of the smoke aerosol upon combustion in a glass chamber. Flexible stainless steel tubing was used to introduce the aerosol into the exposure chamber. The smoke material was filtered (Balston Filter Products, Lexington, Mass.) at the exit port of the chamber so as to remove over 99% of the aerosol before the air flow from the chamber was exhausted through a ceiling vent to air outside the building.

RP/BR Smoke Measurements

Aerosol mass was determined by weighing a filter holder and fiberglass filter before and after exposure to a sampling rate of 1 l/min of smoke drawn from the chamber over each session. Phosphoric acid concentration was measured using an automatic titration system (Radiometer America, Inc., Cleveland, Ohio) and fiberglass filter sample disks. The determinations produced an index of the total phosphorus content (Burton et al., 1982) for each exposure session. Aerosol opacity was measured with an ORNL sensor (Holmberg et al., 1985). Chart records of the relative opacity of smoke during each exposure session were used to continuously monitor smoke concentration and, in conjunction with the aerosol mass measure, to determine the steady-state concentrations achieved during each session. Particle size was measured in terms of mass median aerodynamic diameter (MMAD) and geometric standard deviations (σ_g). A 10-stage quartz-crystal microbalance (QCM) cascade impactor (California Measurements, Inc., Sierra Madre, Calif.) was used to measure particle sizes ranging between 0.10 and 25 μm. Respiratory and contaminant gases (O_2, CO_2, CO, PH_3, and C_6H_{14}) were measured for concentration using industrial analyzer tubes (Gastec Inc., Newark, Calif.). Other measures included temperature, relative humidity, and duration of exposure. All smoke exposure sessions consisted of 60 min of RP/BR burning, with an average of 20 min added to inhalation chamber confinement time to allow for venting before the animals were returned to their home cages.

As indicated in Table 1, exposure concentrations for prairie dogs ranged from 1.2 to 1.4, 3.0 to 3.4, and 4.2 to 7.0 mg/L with the total mass measure for the 2.0, 4.0, and 6.0 mg/L target concentration levels, respectively. Corresponding steady-state concentrations during the exposures achieved median values of 1.8, 4.3, and 5.9 mg/L, respectively (Shumake et al., 1992). For rock dove exposures, total mass concentrations ranged from 2.2 to 2.9 and 4.0 to 5.0 mg/L for the 3.0 and 6.0 mg/L target concentration levels, respectively. These measured concentrations corresponded with median steady-state concentrations of 3.3 and 5.9 mg/L, respectively. For

TABLE 1. Minimum/Maximum in Chamber Measurements Obtained During All Exposures of Prairie Dogs and Rock Doves to Designate Target Concentrations of RP/BR Smoke

	Prairie dogs				Rock doves		
Variable	0.0 mg/L	2.0 mg/L	4.0 mg/L	6.0 mg/L	0.0 mg/L	3.0 mg/L	6.0 mg/L
Aerosol							
Mass (mg)	–1.6–33.0	93.3–114.9	262.1–273.0	321.1–593.3	–4.1–0.7	175.9–209.9	320.3–387.8
Mass concentration (mg/L)	0.0–0.4	1.2–1.4	3.0–3.4	4.2–7.0	0.0–0.5	2.2–2.9	4.0–5.0
Steady-state concentration (mg/L)	NC	1.8–1.8	4.3–4.4	5.6–9.4	NC	2.9–3.4	5.3–6.4
H_3PO_4 (mg)	ND	71.2–85.9	182.8–198.2	234.8–283.9	ND	131.9–159.1	232.4–280.5
H_3PO_4 concentration (mg/L)	ND	1.0–1.1	2.2–2.6	3.0–3.3	ND	1.5–2.2	2.8–3.6
H_3PO_4 as percent of mass	NC	71–76	71–76	48–74	NC	69–76	70–75
Particle size[a]							
Mass median Aerodynamic Diameter (μm)	ND	0.62–0.66	0.82–0.94	NM	ND	0.68–0.89	NM
Respiratory gases							
O_2 (%)	18–21	22–22	22–22	16–22	19–21	19–19	15–22
CO_2 (ppm)	484–726	665–907	968–1089	726–968	387–484	545–641	605–847
Contaminant gases[b]							
CO (ppm)	ND	6–18	7–36	6–36	ND	5–18	ND–36
PH_3 (ppm)	ND	ND	ND–0.1	ND–0.2	ND	ND	ND–0.1
C_6H_{14} (ppm)	ND	ND	ND	ND–121	ND	ND–61	30–73
Exposure/chamber							
Length of exposure (min)	77–100	75–82	77–91	77–88	87	73–81	77–78
Temperature (°C)	19–25	19–22	19–23	20–25	20–22	18–21	20–21
Relative humidity (%)	48–66	54–67	52–64	50–65	48–52	49–60	48–64

Note. NC, not calculated, ND, not detected, NM, not measured.

[a]Determinations of MMAD were made using a graphical analysis procedure (log particle size diameter versus cumulative probability of sampling detections). No particle size measurements were obtained for the filtered air (0.0 mg/L) exposures; small volume samples were insufficient to determine MMAD values for filtered air exposures.

[b]All Gastec analyzer tube values were corrected for 1646 m elevation (i.e., 628 mm Hg) in accordance with the manufacturer's recommendations. The formula for this correction was: Actual tube value × (760 mm Hg)/(628 nm).

all exposures, the percentage of aerosol mass attributable to phosphoric acid ranged in median values from 69 to 76, and this range was expected based on previous studies (Sterner et al., 1988; Moneyhun et al., 1988). Measured MMAD particle sizes were less than 1.0 μm for all of the 2.0, 3.0, and 4.0 mg/L target levels (i.e., in the respirable ranges). Due to rapid saturation of the QCM sensor crystals, however, the 6.0 mg/L concentration particle sizes could not be measured.

For all particle sizes measured, geometric standard deviations of the MMAD sizes ranged from 0.68 to 1.54 μm. For all exposures, the measured oxygen, carbon dioxide, carbon monoxide, and phosphine values were found to be in the safe, normal range. For hexane, however, values as high as 121 ppm for prairie dog exposures and 81 ppm for rock dove exposures were recorded using the analyzer tube detectors. These high recordings were probably anomalous and due to an interaction of other gases in the RP/BR smoke mixture. Quality assurance checks (Moneyhun et al., 1988) indicated that gas chromatography measures of hexane were consistently below 10 ppm. For all exposures, chamber temperatures ranged from 18 to 25°C and relative humidity ranged from 48 to 67%. Chamber confinement periods ranged from 73 to 100 min depending upon the smoke clearance rate from the chamber after the RP/BR product had been extinguished. For both black-tailed prairie dogs and rock doves, these chamber confinement periods varied randomly across the smoke concentration levels and the number of exposure repetitions.

Procedures

Prairie Dogs Initially, 6 groups of prairie dogs received either 2.0, 4.0, or 6.0 mg/L target concentrations of RP/BR aerosol on either 1 or 2 successive daily exposure sessions. Two additional groups received one or two daily exposure sessions to filtered air for comparable time periods. Animals in each group consisted of 3 males and 3 females that had been randomly selected to represent 3 weight range classes for each sex, and all animals were observed for signs of effects for 28 days after the last exposure session. Further details in regard to animal body weights and the procedure used have been published (Shumake et al., 1992).

No pronounced signs of aerosol exposure were observed after these treatments, however, and 4 additional groups were added to the experimental design 4 mo later in order to more thoroughly evaluate potential RP/BR smoke exposure effects. Two of the added groups received either 3 or 4 successive daily exposures to RP/BR aerosol at the 6.0 mg/L target concentration. This was the maximum level that could be generated reliably at 250 L/min air flow rate in the exposure chamber. The two remaining groups received filtered air exposure sessions for comparable time periods. One of the filtered air groups was given "regular handling," with each animal restrained a few minutes for respiration checks using a stethoscope before and after exercise. The other filtered air group was not restrained, in order to

assess confounding stress effects that could have been introduced by the restraining procedures. Animals for these added groups were also randomly selected with a procedure that gave equal representation to three weight range classes for each sex. This was accomplished by rank-ordering the animals of each sex according to body weight. Then three arbitrary weight classes were set up for each sex: 947–1116 g, 1170–1244 g, and 1279–1516 g for males, and 875–933 g, 1012–1054 g, and 1071–1276 g for females. One male and one female prairie dog were randomly selected from each of the three weight range classes (i.e., a total of three males and three females), and all animals thus selected were assigned to one of the four treatment groups.

For all prairie dog groups, quantitative toxic sign measures (body weight and water consumption) were conducted with the same experimental paradigm. This involved a 7-day preexposure phase (baseline), a 1- to 4-day exposure phase, and a 28-day postexposure phase. Measures were taken daily for the first 7 days postexposure, followed by measures taken every third day. The daily water consumption of each prairie dog was measured by subtracting the pre- and postweight (nearest gram) of each animal's water bottle after approximately 19–26 h, with time of water availability recorded to nearest minute between 0900 and 1400 MST. Bottles were removed, weighed, rinsed, refilled, reweighed, and reattached to each animal's respective cage at the end of each measurement session. Differences in the time of water availability were adjusted for a 24-h time base using the formula:

$$\text{24-h Consumption} = \frac{24 \times (\text{measured water drunk in grams})}{(\text{duration of availability in hours})}$$

These time-adjusted water consumption values were used to remove potential bias in the data related to circadian rhythm effects upon drinking and other activities (Binkley, 1990; Sterner, 1993).

The daily or session (postexposure) body weight of each prairie dog was recorded (nearest gram) using an electronic balance (model PE 3600; Mettler Corp., Hightstown, N.J.). Other qualitative signs of toxicity were evaluated concurrently on these same animal groups, and results have been previously reported (Shumake et al., 1992).

Rock Doves A group of 48 birds (24 of each sex) was used for the study. Sex of each bird was determined by a cloacal examination (Miller & Wagner, 1955). Verification of sexing was based on gross necropsy at the end of the study period. From three weight class ranges for each sex, the animals were randomly assigned to eight treatment groups each consisting of three males and three females. For further details in regard to the selection procedure and body weight ranges, see Shumake et al. (1992).

Six of the groups received either 1, 2, or 3 successive daily exposures to RP/BR aerosol target concentrations of 3.0 and 6.0 mg/L. The remaining 2 groups received either 4 successive daily exposures to RP/BR aerosol at the 6.0 mg/L target concentration or 4 daily exposures to filtered air (0.0 mg/L).

Again, all smoke exposure sessions involved 60 min of RP/BR burning, with chamber confinement periods extending 13–27 min beyond this to allow venting of the inhalation chamber.

The same experimental paradigm previously described for the prairie dog studies was used for the rock dove study. The daily water consumption of each dove was measured as the difference in milliliters of water removed from drinker tubes on the individual cages during the measurement period. Time of water availability (19–26 h) was also recorded to the nearest minute. The waterline (meniscus) was marked with a small rubber band after each tube was filled and affixed to each bird's respective cage. During each measurement session, a ruled scale in milliliters of volume was held next to each tube and the difference between the rubber band and the new waterline (meniscus) determined. Tubes were measured, removed, rinsed, refilled, reattached, and banded at the end of each examination session. Differences in the time of water availability were adjusted for a 24-h time base using the same formula as applied to the prairie dog data. The daily or session (postexposure) body weight of each rock dove was recorded (nearest gram) using an electronic balance (model PE-3600; Mettler Corp., Hightstown, N.J.).

Data Analyses For the prairie dog data, to test hypotheses related to smoke concentration and gender effects, data for the last daily session of the preexposure phase (day 0) were compared with data for 14 sessions during the postexposure phase (days 1–7, 10, 13, 16, 19, 22, 25, 28), in a design that involved a 4 concentrations (0.0, 2.0, 4.0, 6.0 mg/L) × 2 exposures (1 or 2) × 2 sexes × 15 days analysis of variance (ANOVA) on the initially tested 6 groups. The added groups were not evaluated in the same design because of the 4-mo time interval that led to an overall increase in their body weights. For each measure, a second ANOVA was used to test the null hypotheses related to the number of exposures effects. This consisted of a 4-factor repeated measures design that involved 2 concentrations (0.0 and 6.0 mg/L) × 3 exposures (1, 2, or 4) × 2 sexes × 15 days, with days treated as a repeated-measures factor (Winer, 1971). All ANOVAs were computed using the general linear hypothesis model (PROC GLM) program of the SAS package of programs (SAS Institute, Inc., 1985) and Type III sums of squares to test for statistical significance.

For statistical analyses of the rock dove data, two similar repeated-measures ANOVA designs (Winer, 1971) were used to analyze the water consumption and body weight data. Data for the last day of the preexposure phase were compared with the data for 14 daily sessions of the postexposure phase, again using the PROC GLM of the SAS package of programs (SAS Institute, Inc., 1985) and Type III sums of squares. The first ANOVA consisted of a 2 concentrations (3.0, 6.0 mg/L) × 3 exposures (1, 2, or 3) × 2 sexes × 15 days design. The second ANOVA consisted of a 2 concentrations (0.0, 6.0 mg/L) × 2 sexes × 15 days design. Where significant ($p < .05$) effects were found in each ANOVA, post hoc Duncan multiple range tests (Waller & Duncan, 1969) were used for pairwise comparison of all means.

RESULTS

Black-Tailed Prairie Dogs

Body Weight A summary of the significant main and interaction effects is shown in Table 2. All comparisons were based on group means of the preexposure body weights as compared to group means of the body weights observed postexposure (e.g., days 1–7, 10, 13, etc.). The four-factor data analysis comparing the effects of concentration, exposure repetition, sex, and days detected three significant effects: sex, day, and day × exposure. The second data analysis comparing concentration, sex, and days also detected three significant effects: sex, day, and exposure. Each effect

TABLE 2. Significant Main and Interaction Effects Detected by Two ANOVAs on Body Weight and Water Consumption in Different Prairie Dog Groups

Source	df	*F*-Ratio	$p \leq$	Analysis type	Null hypotheses[c] rejected
		Body weight			
Sex	1,34	7.09	.012	Four-factor[a]	(1)[d]
Day	14,448	99.48	.0001		
Day × exposure	14,448	1.69	.054		(6)
Sex	1,30	10.01	.0035		(1)[d]
Day	14,420	62.78	.0001		
Exposure	2,30	5.39	.01	Four-factor[b]	(5)[e]
		Water consumption			
Day	14,444	7.64	.0001	Four-factor[a]	
Day × concentration	42,444	2.26	.0001		(4)
Day × exposure	14,444	2.41	.0029		(6)
Day × sex	14,444	2.18	.0079		(2)
Day × concentration × exposure	42,444	2.09	.0001		(4)(6)
Day	14,417	8.03	.0001	Four-factor[b]	
Day × concentration	14,417	4.25	.0001		(4)
Day × exposure	28,417	2.70	.0001		(6)

[a]Four concentrations (0.0, 2.0, 4.0, 6.0 mg/L) × 2 exposure repetition (1, 2) × 2 sexes × 15 days (1 pre- and 14 postexposure).

[b]Two concentrations (0.0, 6.0 mg/L) × 3 exposure repetition (1, 2, 4) × 2 sexes × 15 days (1 pre- and 14 postexposure).

[c](1) Males and females were equally affected.
(2) Males and females were affected for the same period.
(3) All concentrations produced equal effects.
(4) All concentrations produced effects for the same period.
(5) Single and multiple exposures produced equal effects.
(6) Single and multiple exposures produced effects for the same period.

[d]Procedure artifact (male and female groups were not equal on preexposure day).

[e]Procedure artifact (4-exposure group was heavier due to extra 4-mo holding period before exposure to smoke).

was interpreted within the context of the three hypothesis pairs that considered gender, concentration, and exposure as factors that could alter either the magnitude or durations of the smoke exposure effects upon mean body weights.

Significant main effects for sex ($p < .012$; $p < .0035$) were detected in each analysis. However, based upon a comparison of the initial mean weights of male versus female animals, these results were expected and did not result from smoke exposure treatments. Mean male weights were 11.2% greater than the female weights (1076.3 g vs. 955.7 g, respectively).

No significant main or interaction effects with concentration as a component part were detected in either analysis. The hypotheses that neither the magnitude nor the duration of smoke exposure effects upon body weight would be affected by concentration were therefore both accepted.

In the four-factor analysis, the day x exposure effect ($p < .054$) achieved a borderline level of significance. Duncan multiple range tests among the treatment means indicated that the 1- and 2-exposure groups were significantly different on the last preexposure day with the 2-exposure group being 2.7% heavier. This difference persisted throughout day 10 postexposure. By day 25, however, the 2-exposure group was only 1.2% heavier (nonsignificant). The effect suggested that animals in the single exposure group gained weight slightly faster than those in the two-exposure group late in postexposure.

The second (four-factor) analysis detected exposure ($p < .01$) as a significant main effect. This effect, however, was expected, and was generated as an artifact of the animal holding procedure. The 4-exposure group compared to the 1- and 2-exposure groups had been held in a colony for 4 mo longer prior to their smoke exposures and had consequently gained a significant mean increase in weight.

Water Consumption Significant main and interaction effects detected by each data analysis are listed in Table 2. Day main effects as well as several interactions were detected for both analyses. These are described separately within the limits of the three pairs of null hypotheses regarding gender, concentration, and exposure repetition as factors affecting water consumption after smoke exposure.

Only one day x sex interaction effect ($p < .0079$) was detected by the four-factor analysis. Multiple comparisons indicated that males and females were not different in their mean water consumption levels on the preexposure day and that they remained this way through day 19. However, on days 22 and 28, the males drank significantly more water (approximately 25%) than the females during this last week of the test.

Concentration was not significant in either data analysis as á main effect. The four-way analysis, however, detected a day x concentration x exposure interaction ($p < .0001$) that reflected the operation of two two-way effects: day x concentration ($p < .0001$) and day x exposure ($p < .0029$). For the day x concentration effect, multiple range tests indicated a

significant increase (21%) in mean water consumption by the 6.0 mg/L group compared to controls late in postexposure (days 10–28). In contrast, the 2.0 and 4.0 mg/L groups drank significantly less (33%) than the control group later in postexposure days (22–28). For the day × exposure effect, multiple range tests indicated two subeffects. First, the 1-exposure group animals consumed less than their preexposure mean levels for the first 7 days postexposure; the 2-exposure group animals were only significantly suppressed in intake levels for the first 3 days postexposure. Second, these two groups reversed their mean consumption levels on the last day of post-exposure.

The second analysis yielded only a day × concentration ($p < .0001$) effect. Multiple range tests indicated that the 6.0 mg/L animals showed significant depression of their mean water consumption on the first 2 days postexposure. However, late in postexposure, on days 10–28, these animals drank significantly more water when compared with the 0.0 mg/L animals.

Figure 1 graphically shows this day × concentration interaction effect. As depicted, the groups do not differ statistically on the preexposure day (day 0). Immediately after exposure, however, the 6.0 mg/L group showed an initial drop (approximately 37%), and then the animals rapidly increased their mean water intake to normal levels through day 7. On day 10, and on days 19–28, however, these animals drank more water (approximately 31%) compared to the 0.0 mg/L group. The day effect ($p < .0001$) in Table 2 indicated an overall increase in consumption during the later days of post-exposure, but this was, of course, largely due to the elevated intakes of the 6.0 mg/L animals.

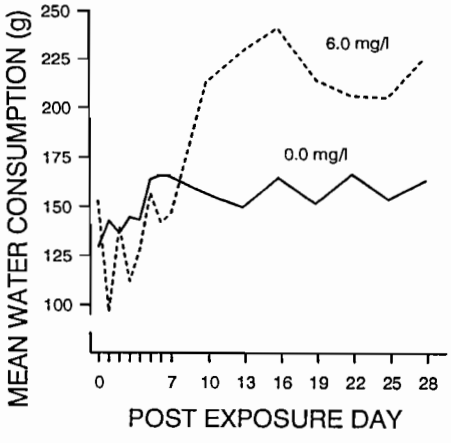

FIGURE 1. Mean water consumption by prairie dogs as a function of time after exposure for the 0.0 versus 6.0 mg/L RP/BR smoke exposure levels. The 1, 2, and 4 daily smoke exposure groups have been combined (n = 18/group) to indicate the two-way interaction effect. Day 0 is the last preexposure day, and days 1–28 are the mean water intake values on the days following the last daily exposure to RP/BR smoke.

As already indicated, significant day × concentration × exposure ($p \leq .0001$) and day × exposure ($p \leq .0029$) interaction effects on water consumption were found in the four-way analysis. For the day × exposure interaction effect, multiple comparisons revealed that the 1-exposure group was significantly depressed for 7 days and the 2-exposure group depressed for only 3 days postexposure. On days 3–25, however, the 2-exposure group animal drank at levels significantly above their preexposure day level.

The second data analysis only detected a day × exposure effect ($p < .0001$). When further analyzed graphically along with multiple mean comparisons, the four-exposure animals as compared to the one- and two-exposure animals were found to have significantly depressed water intake levels late in postexposure (days 10–28). This result thus supported an alternate hypothesis stating that multiple-exposure animals would be affected for a longer period during postexposure.

Rock Doves

Body Weight Significant main and interaction effects upon body weight are shown in Table 3. The four-factor data analysis compared effects of smoke concentration, exposure repetitions, sex, and days. Day as a main effect and four interaction terms each containing day as a component part were detected. Likewise, the three-factor data analyses compared smoke concentration, sex, and day effects. Day was again found to be significant along with three interaction terms each containing day as a component part. Detected effects were evaluated as they related to the initially posed null hypotheses regarding gender, concentration, and number of RP/BR smoke exposures.

Two interactions containing sex as a component part were detected by the four-factor analysis: day × concentration × sex × exposures ($p < .0029$) and day × concentration × sex ($p < .0003$). The four-way interaction is presented graphically in Figure 2. As shown, this effect (and a large proportion of the three-way effect) stems from the fact that all males given 2 exposure repetitions at the 6.0 mg/L level had a drastic and steady decline in mean body weight on days 1–6 postexposure (center graph). By day 7, all males in this group were dead. Females in this group, on the other hand, survived through the 28 days postexposure and showed no significant decline in mean body weight. Another partial account of the four-way effect can be seen in the three-exposure group (bottom graph). In this case, the 6.0 mg/L males showed sustained depression of mean body weight on days 10–28 when compared to the 3.0 mg/L males. The corresponding female groups, however, showed no significant differences in mean body weight during this period. The 6.0 mg/L females relative to the 3.0 mg/L females did, however, show significantly more decline from their initial mean weights (day 0) during the first week postexposure (days 1–7). The four-way interaction effect was therefore mainly generated by a gender difference interacting strongly with the other three factors: concentration, exposure and day.

TABLE 3. Significant Main and Interaction Effects Detected by Two ANOVAs on Body Weight and Water Consumption in Different Rock Dove Groups

Source	df	*F*-Ratio	$p \leq$	Analysis type	Null hypotheses[c] rejected
	Body weight				
Day	14,271	39.78	.0001	Four-factor[a]	
Day × concentration	14,271	6.01	.0001		(4)
Day × exposure	28,271	3.19	.0001		(6)
Day × concentration × sex	14,271	3.01	.0003		(2), (4)
Day × concentration × sex × exposure	20,271	2.18	.0029		(2), (4), (6)
Day	14,90	5.19	.001		
Day × concentration	14,90	2.38	.0072	Three-factor[b]	(4)
Day × sex	14,90	3.43	.0002		(2)
Day × concentration × sex	14,90	1.84	.0441		(2), (4)
	Water consumption				
Day	14,271	12.24	.0001	Four-factor[a]	
Day × concentration	14,271	2.65	.0012		(4)
Day × exposure	28,271	2.18	.0008		(6)
Day × concentration × exposure	28,271	2.07	.0017		(4), (6)
Day	14,90	3.54	.0001	Three-factor[b]	

[a]Two concentrations (3.0, 6.0 mg/L) × 3 exposure repetition (1, 2, 3) × 2 sexes × 15 days (1 pre- and 14 postexposure).

[b]Two concentrations (0.0, 6.0 mg/L) × 2 sexes × 15 days (1 pre- and 14 postexposure).

[c](1) Males and females were equally affected.
(2) Males and females were affected for the same period.
(3) All concentrations produced equal effects.
(4) All concentrations produced effects for the same period.
(5) Single and multiple exposures produced equal effects.
(6) Single and multiple exposures produced effects for the same period.

The second, three-factor data analysis detected two interaction terms that each contained sex as a component part: day × concentration × sex ($p < .0441$) and day × sex ($p < .0002$). Figure 3 is a graphical representation of this three-way effect. Individual mean comparisons further revealed that the 6.0 mg/L males were significantly depressed in mean body weight compared to the 0.0 mg/L males on all postexposure days except days 3 and 4. These groups were not significantly different on the preexposure day. The 6.0 mg/L and 0.0 mg/L females were not significantly different from one another on the preexposure day and all of the postexposure days except for days 25 and 28, when the 6.0 mg/L group means were lower. Again, gender was found to have a significant effect on the duration of body weight depression postexposure. This was also reflected in the day × sex effect ($p \leq .0002$). Females showed a slight gain in mean weight over days after a minor decline for the first day of postexposure. The males, however, mainly due to effects

three-exposure group showed no significant reduction in intake compared to preexposure. Also, all 3 exposure repetition groups were significantly different from one another on days 7–25, with amount of water consumption being directly related to number of exposure repetitions.

The second, three-way data analysis detected only one significant effect, day ($p < .0001$). Multiple range tests indicated that more water intake occurred on days 10, 19, and 28 than on the other postexposure days. This represented a tendency for all doves to increase their daily water consumption over the 28 days of postexposure.

DISCUSSION AND CONCLUSIONS

As indicated in a previous report (Shumake et al., 1992) on qualitative signs associated with RP/BR smoke exposures in prairie dogs and rock doves, the 1.8–5.9 mg/L steady-state exposure levels in the current report on the same animals far exceeded normal field exposure ranges (Garvey et al., 1981; Aranyi et al., 1988). However, one of the main objectives of the exposure study was to define a safe, sublethal range for use in later studies (Johns et al., 1992; Sterner, 1993) on the general activity, startle response, pulmonary function, and hematological effects in these two species. Within the confines of these restrictions and objectives, several reliable effects related to body weight loss and water consumption changes were revealed in the present study, and these warrant further explanation and clarification.

Rock doves were much more severely affected by body weight losses after RP/BR smoke exposure than were prairie dogs. Prairie dogs exposed to RP/BR aerosol generally showed only a 1-day loss in weight gain, followed by a rapid recovery to preexposure levels by day 3 postexposure. Also, the one-exposure groups gained weight slightly faster than the two-exposure groups during post exposure. Rock doves, on the other hand, showed a sex difference in their body weight loss after RP/BR aerosol exposures, in agreement with previously reported results for albino rats (Aranyi, 1983). When compared to the female groups, males had much greater losses of body weights, showed higher mortality, and survivors did not recover to their preexposure mean body weights for the duration of the 28-day postexposure period. The 6.0 mg/L groups, compared to the 3.0 mg/L groups, were more severely depressed and did not return to their preexposure body weight levels until 22 days postexposure.

Water consumption measures for the two species yielded results in partial agreement. In prairie dogs, groups given RP/BR aerosol exposures showed a short but sharp depression in water intake, and then their intakes significantly increased late in postexposure (days 10–28) when compared to preexposure intakes. The elevated water consumption was significantly higher in the two-exposure compared to the single exposure group. The second four-factor analysis, however, where a four-exposure group was added, for comparison, did not confirm this exposure effect. This analysis did confirm that those rock

dove groups that received multiple as opposed to single exposures showed more water consumption late in postexposure (days 7–25).

These protracted periods of elevated water consumption that were consistently observed for both species could have been related to RP/BR smoke-induced pulmonary irritation, inflammation, and edema (Burton et al., 1982). These pathological indications were not, however, firmly verified upon necropsy in this study in either species. A detailed description of the complete necropsy results is reported elsewhere (Shumake et al., 1992). Due to the nature of the study, however, the necropsy data were not taken until 30 postexposure days had elapsed, and tissue healing could have occurred during this period. Measures of core body temperature and urine excretion rates taken daily relative to water intakes could be used to help clarify these and other interpretations in future studies.

Physical obstructions in the form of exudate or mucus in the bronchi, larynx, and epiglottis were noted in necropsy examinations on rock doves that succumbed to the RP/BR smoke exposure treatments. Reduced ciliary motility has been previously indicated as a pulmonary effect of sulfuric acid aerosol exposure (Phalen, 1984), and RP/BR smoke exposures can lead to lowered immunological responses (Aranyi et al., 1988) as a respiratory effect in albino rats. For some animals, at least, these factors could have required greater energy expenditure and exertion to maintain adequate respiration. Increased respiration rates and respiratory exchange ratios were, in fact, found in rock doves postexposure to 4.0 mg/L smoke concentrations in later studies (Johns et al., 1992). These factors could have then caused an increase in the need for higher water intake levels.

Day × concentration interaction effects on water consumption were significant for both species also. However, these results were not in the same direction. The highest consumption levels were found in prairie dogs in the 6.0 mg/L group late in postexposure (days 10–28), with the 2.0 and 4.0 mg/L groups showing depressed consumption levels compared with the filtered-air exposed group during this interval. The rock dove 3.0 mg/L group, in contrast, showed higher consumption levels in late postexposure during days 10–28 when compared to the 6.0 mg/L group. These paradoxical findings were assumed to have been partially generated by the high rock dove mortality at the 6.0 mg/L level. Those doves that might have shown even more elevated water consumption levels late in postexposure had died within wk 1 postexposure. Prairie dog data were not limited by this mortality factor.

As indicated in our previous report (Shumake et al., 1992), a more refined analysis of water consumption and body weight effects postexposure to RP/BR smoke would involve taking lung tissue or blood biopsies from the animals to screen for pulmonary disease potential prior to exposure. This procedure would increase the likelihood of detection of more subtle RP/BR aerosol effects by possibly reducing variance in the air-control exposed animals.

Despite this limitation, however, RP/BR smoke has been shown to be capable of producing long-term (28-day) significantly elevated water con-

sumption in both species, and severe, protracted weight loss effects in male rock doves. Smoke concentration level appeared to be the main controlling factor for these observed effects, rather than number of exposure repetitions. This overall finding is in agreement with a previous investigation (Aranyi et al., 1988) on albino rats.

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